Characterization of Unifloral Honeys from Kashmir Valley of India According to their Physicochemical Parameters

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Abstract—*The characterization of four unifloral honey samples from Kashmir valley of India (apple, cherry, saffron and Plectranthus rugosus) was carried out on the basis of some common physicochemical parameters (pH, free acidity, lactone acidity, total acidity, electrical conductivity, total soluble solids, total solids, specific gravity, refractive index and color). The results obtained, indicates that all the four analyzed varieties of honey met the criteria set by the European Commission Regulation and the revised codex standard for honey which proves that the honey from Kashmir valley is high quality honey. The source of honey had a significant effect ($p<0.05$) on all physicochemical parameters.*

Keywords: *Apple, saffron, cherry, Plectranthus rugosus, honey, Kashmir, physicochemical*

1. INTRODUCTION

Honey is a natural sweet and complex product formed by honeybees from nectar or honeydew which has gained much attention of scientists and researchers in the last few years. The past studies have proved that honey posses antioxidant, antihypertensive, antibacterial, anti-fungal and anti-inflammatory effects [1].The composition of honey depends on the plant species visited by the honeybees, environmental, processing and storage conditions [2-3]. Honey generally is composed of sugars mainly monosaccharides while disaccharides, oligosaccharides and polysaccharides are also present in trace amounts [3]. It also contains enzymes (glucose oxidase, diastase, invertase, catalase and peroxidase) and other bioactive constituents such as phenols, flavonoids, organic acids, minerals, vitamins, amino acids etc which contribute to the antioxidant activity of honey [4, 6-7]. Various literatures on physico-chemical analysis is available all over the globe including India but no study regarding the honey available from such sources from Kashmir Valley of India is available yet so the main aim of the present study is to characterize the physicochemical parameters from four different varieties of honey from Kashmir valley of India.

2. MATERIAL AND METHODS

2.1. Honey sample collection

All the fresh and raw honey samples viz. apple (*Malus domestica*), cherry (*Prunus avium*), saffron (*Crocus sativus*) and wild bush (*Plectranthus rugosus*) were collected from local beekeepers of Pulwama, Pampore and Srinagar areas of Kashmir valley of India and were packed and sealed in glass bottles during September 2012 to November 2013. The honey samples were stored at 4 $^{\circ}$C until further analysis.

2.2. Physicochemical analysis

All physico-chemical characteristics: pH, total soluble solids and total solids, free acidity, lactone acidity, total acidity, specific gravity, refractive index, electrical conductivity and color (mmpfund) were performed in triplicates as per the methods established by AOAC (2012) and International Honey Commission (2009) [8-9].

2.2.1. pH

pH was measured with a combined pH glass electrode connected to pH meter Basic 20, in a solution prepared with 10 g of honey in 75 ml of distilled water. The instrument was calibrated with standard buffer solutions of pH 7 and pH 4, prior to measuring the pH of samples.

2.2.2. Free acidity, lactone acidity and total acidity

Free, lactone and total acidities were determined by a titrimetric method as follows: the addition of 0.05M NaOH is stopped at pH 8.5 (free acidity), followed by addition of 10 ml volume containing 0.05M NaOH and, without delay is back titrated with 0.05 M HCl to pH 8.3 (lactone acidity). Total acidity results are obtained by adding free and lactone acidities. All the results were done in triplicates and were expressed as meq/kg.

2.2.3. Electrical conductivity

Electrical conductivity measures the electrical resistance which was measured by a conductimeter. The sample solution was prepared using milli Q water (20% w/v). Results were expressed in mS/cm [10].

2.2.4. Total Soluble Solids (TSS) & Total Solids (TS)

The total soluble solids of the honey samples were measured by refractometry using an Atago hand held refractometer and the results were expressed in °Brix. All the measurements were done at ambient temperature and the readings were corrected for a standard temperature of 20°C by adding the correction factor of 0.00023/°C [8]. The total solid content (%) in the honey samples was calculated by using the formula: TS (%) = 100–moisture content [11]. Refractive index and specific gravity were obtained as per Wedmore's Table [12]

2.2.5. Color

Honey samples were heated to 50°C to dissolve sugar crystals, and the color was determined by using Hach Lange DR6000 UV-VIS spectrophotometer. The absorbance of a 50% honey solution (w/v) was determined at 635 nm. The honeys were classified according to the Pfund scale after conversion of the absorbance values: mmPfund = -38.70 + 371.39 × Abs [13].

3. STATISTICAL ANALYSIS

All the honey samples were analyzed in triplicates. Mean, standard deviation and correlation were obtained by using Microsoft excel 2007. One-way analysis of variance (ANOVA) was used to find the significant differences among the means followed by Duncan's multiple range test (DMRT) ($P < 0.05$).

4. RESULT AND DISCUSSION

All the honey samples tested were found to be acidic in nature as their pH values were ranged from 3.01 to 4.35 and were significantly different ($P < 0.05$) (Table 1). In general, honey is acidic in nature irrespective of its variable geographical origin. The pH values were in agreement with the results of Indian commercial honeys, Algerian, Brazilian, Spanish and Turkish honeys whose pH values have been found to vary between 3.7 to 4.4, 3.49 to 4.53, 3.10 to 4.05, 3.63 to 5.01 and 3.67 to 4.57, respectively [14-17].The acidity of honey is due to presence of organic acids in the form of gluconic acid, citric acid, pyruvic acid in equilibrium with lactones and ions. A high positive correlation was observed between pH and electrical conductivity (r=0.72) as shown in Table 2. Results in Table 1 indicated that free acidity ranged 19.65 to 38.03 meq/kg while lactone acidity and total acidity ranged from 5.82 to 8.22 meq/kg and 26.80 to 46.17 meq/kg respectively. The free acidity of all the samples analyzed was found within the corresponding limits of as described by Codex Almentarious Commission (2001) which proves that there was no undesirable fermentation in honey samples [18]. Similar results were published by Chakir et al. (2011), Chefrour et al. (2009) and Zerrouk et al. (2011) [19-21]. Strong and positive correlations were found between total acidity and free acidity (r=0.99), total acidity and lactone acidity (r=0.73). ^{o}Brix values (TSS) were ranged from 78.93 to 80.7 while the refractive index and specific gravity of the samples ranged from 1.4881 to 1.4921 and 1.4076 to 1.4183 respectively. Generally the refractive index increases with the increase in the solid content. The total solid content of the samples were in a range of 80.6 % to 82.33 %, which were quiet similar to the findings as reported by Saxena et al, (2010) [14]. As shown in Table 2, both TS and TSS show positive and strong correlation with specific gravity (r=0.90, r=0.95). Electrical conductivity depends on the mineral content of honey and it has been can be considered to be a valid criterion for differentiation between honeydew and nectar honeys [22]. The electrical conductivity of our tested honey samples ranged from 0.23 to 0.69 mS/cm. As per Codex Alimentarius (2001) guidelines is concerned, the electrical conductivity value for the nectar honey should be less than 0.80 mS/cm [18]. The electrical conductivity data of honey from Kashmir valley are consistent with previously reported values of European unifloral honeys, and Serbian unifloral honey [23-26].The color of honey is one of the main factor that is responsible for determining its price in the world market, and its acceptability by the consumers. The color characteristics of the honey samples are presented in Table 1. According to the USDA, honey samples with Pfund values less than 8mm are classified as "water white" while those with greater than 114 are classified as "dark amber" [27]. Based on this classification, the apple variety (135.18mm) was classified as dark amber, cherry honey (110.33mm) as amber, *Plectranthus rugosus*

Table 1: Physico-chemical characteristics of honey samples assessed by Duncan's multiple range test.

Parameter	saffron	apple	cherry	plectranthus rugosus
pH	3.57 ± 0.32b	4.35 ± 0.22a	3.01 ± 0.18c	3.42 ± 0.28b
Free acidity (meq/kg)	32.64±2.24b	19.65±2.11d	38.03±1.95a	23.67±2.03c
Lactone acidity (meq/kg)	8.22±0.52b	7.15±0.31b	8.14±0.87b	5.82±0.70b
Total acidity (meq/kg)	40.87±1.97b	26.80±2.40c	46.17±2.30a	29.49±2.72c
Total soluble solids (°B)	78.93±0.40b	79.3±0.3b	80.7±0.3a	80.43±0.11a
Total solids (%)	80.6±0.04b	81.0±0.3b	82.33±0.8a	82.03±0.11a

Color (mmpfund)	37.36±0.83d	135.18±0.84a	110.33±1.5b	73.63±0.84c
Specific gravity	1.4076±0.002b	1.4101±0.001b	1.4183±0.003a	1.4176±0.000a
Refractive index	1.4881±0.0001b	1.4889±0.0001b	1.4921±0.0001a	1.4915±0.0001a
Electrical Conductivity (mS/cm)	0.23±0.04c	0.69±0.07a	0.34±0.04b	0.29±0.02bc

Results are expressed as mean values±standard deviations. Means in a row with same superscripts are not significantly different (P<0.05)

Table 2: Correlation among different physicochemical and color parameters (Pearson correlation coefficients P<0.05).

	Free acidity	**Lactone acidity**	**Total acidity**	**pH**	**TS**	**TSS**	**Color**	**Specific Gravity**	**Electric Conductivity**	**Refractive Index**
Free acidity	1.00	-	-	-	-	-	-	-	-	-
Lactone acidity	0.65	1.00	-	-	-	-	-	-	-	-
Total acidity	0.99	0.73	1.00	-	-	-	-	-	-	-
pH	-0.74	-0.16	-0.69	1.00	-	-	-	-	-	-
TS	0.23	-0.13	0.20	-0.42	1.00	-	-	-	-	-
TSS	0.28	-0.21	0.22	-0.49	0.90	1.00	-	-	-	-
Color	-0.31	-0.08	-0.30	0.33	0.30	0.27	1.00	-	-	-
Specific Gravity	0.22	-0.34	0.16	-0.57	0.90	0.95	0.27	1.00	-	-
Electric Conductivity	-0.63	-0.16	-0.59	0.72	-0.17	-0.23	0.83	-0.21	1.00	-
Refractive Index	0.34	-0.24	0.27	-0.64	0.87	0.97	0.22	0.98	-0.30	1.00

honey (73.63mm) as light amber while saffron (37.36mm) as extra light amber. Our results were consistent with those reported for New Zealand honey, Malaysian honey and Algerian honey [28-30]. Color depends on the floral origin and the minerals content. Positive correlation was found between color and electrical conductivity (r= 0.72) (Table 2). The color of honey is closely related to its chemical composition, primarily to the presence of pigments such as chlorophylls, carotenoids, flavonoids and polyphenols [31].

5. CONCLUSION

The results obtained show high quality of honey, indicating that all four tested unifloral varieties of honey meet the criteria set by the Council Directive 2001/ 110/EC and the Revised Codex Standard for Honey, Codex STAN 12- 1981 regarding the studied physicochemical properties. Although the investigated physicochemical parameters reveal the chemical composition of honey but they are not sufficient to define the geographical or botanical origin of honey from Kashmir valley.

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